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CHEMICAL EXTRACTION TECHNIQUES TO FREE FOSSIL SILICOFLAGELLATES FROM MARINE SEDIMENTARY ROCKS

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Abstract. Techniques of extracting fossil silicoflagellates and other siliceous microfossils from marine sedimentary rocks are described. The extraction is achieved by dissolving and chemically disaggregating all the rock (or the cementing agents of the rocks) except the siliceous fraction, and by removing the nonfossiliferous fraction so that the fossils will not be hidden.

Introduction

This paper, while written primarily for micropaleontologists who work with silicoflagellates,[1] can also be used by those who study other siliceous microfossils such as diatoms, radiolarians, ebriata, sponge spicules, and rocellids.

It is our intent that this paper be a useful working tool for micropaleontologists who are not either chemists or geochemists. It is not intended to be a monographic study, recording, comparing, and evaluating other techniques. Hence

[1] Silicoflagellates are defined here as marine, planktonic Mastigophora (Protozoa) with a pseudopodium, a flagellum, and a skeleton of hollow siliceous rods. These organisms also contain color pigment organelles and therefore are treated by some workers as plants (Algae) and by others as an animal-plant group (Protista).

no long list of prior papers dealing with this subject is cited. Papers omitted from our much abridged list have not been judged less worthy by us. Their omission results simply from our wish to present a short list. The reader who wishes to compare other techniques is referred to the following papers and their bibliographies: Mann (1922), Hustedt (1958), Hanna (1937), Burma (1965), Gray (1965), and Schopf (1965). The first four workers have techniques that are similar to each other, and similar to our techniques. However, there are sufficient differences between our methodology and the earlier procedures to justify making our data available to those who work with siliceous microfossils. Gray and Schopf deal with pollen, but their papers include some techniques and procedures that are transferable to the study of siliceous microfossils.

Rocks that contain fossil silicoflagellates vary from friable (*e.g.* pure diatomite) to well indurated (*e.g.* limestone). The techniques presented here can be used for all marine sedimentary rocks except those which have essentially the same chemical composition as silicoflagellates. Examples of rocks for which these techniques will not work are chert and jasper. Siliceous microfossils in these kinds of rocks must be studied by thin sections and polished surfaces. The reader is referred to any standard petrographic text for these techniques. They are not included here because it has been our experience that the study of silicoflagellates and diatoms by thin sections and polished surfaces is at best not satisfactory and, in most cases, not worth the effort.

Objectives

The purpose of this paper is to describe our techniques of extracting fossil silicoflagellates and other siliceous microfossils from marine sedimentary rocks. If the procedures given here are followed, all individual specimens of these microorganisms will be relatively clean (i.e., no large particle of debris will be cemented to the specimens).

These results are achieved by: (1) dissolving and chemically disaggregating all the rock (or all the cementing agent of the rock) except the siliceous fraction of the rock and the siliceous microfossils in the rock; and (2) decanting away the nonfossiliferous part of the rock so that silicoflagellates will not be hidden by these materials. In general only acids and other chemicals that do not attack siliceous microfossils are used, the one exception being the use of sodium hydroxide (NaOH), which can dissolve silicoflagellates. However we use it as a dilute solution (0.25–1.0 N) and only for a relatively short time. No appreciable damage to silicoflagellates or diatoms by such use of sodium hydroxide has been observed.

Deep-sea core samples and outcrop samples. There is such a wide variation in the lithology, mineralogy, and chemistry of marine sedimentary rocks containing fossil silicoflagellates that no one simple chemical extraction (or

simple generalized method) will be applicable to all marine sedimentary rocks. However, one generalization is possible: most deep-sea core samples are more easily prepared chemically, as compared with most older Cenozoic samples taken from outcrops of well indurated rock. Therefore many steps of the procedure presented here may not be necessary for deep-sea core samples. In contrast, a large number of important New Zealand Early Tertiary samples taken from outcrops consisting of well indurated rock and cemented by complex compounds, have taken as long as two weeks of continuous work per sample to prepare.

Number of samples to be processed at one time. If the worker has adequate experience and adequate equipment, and no problems develop, then six samples can be processed as a batch—at one time. However, at the sulfuric acid fuming[2] step only one sample at a time can be worked. Frequently we find that three or four samples are all that the most experienced worker can handle at one time. Again, this excludes the sulfuric acid fuming step, when but one sample should be handled. Furthermore it is not uncommon that one sample could be such a continous source of trouble that it should be processed alone. Beginners, for reasons of safety and efficiency, should work with only one sample at a time.

Directions and Comments

Read each set of directions completely before you start to do each step.

Rubber gloves, plastic apron, face mask, and goggles should be used at all times while handling acids or sodium hydroxide solutions or hydrogen peroxide solutions. Use a fume hood when working with beakers containing acids.

1. Place the rock sample which is to be prepared on a layer of newspapers. With a clean ice pick break a piece from each large fragment of the rock sample so that the material to be treated will be representative of the whole sample. The remaining untreated portion of the rock sample should be preserved for future study. Reduce all the pieces to about one-fourth inch in size with the ice pick. The maximum amount of the sample to be treated should be about 100 grams.

   After each sampling has been made, carefully roll up and discard the newspapers, and clean the ice pick. This procedure will prevent contamination of one sample by another.

2. Label a clean 1000-ml. Pyrex beaker with sample number. Place about 100 grams of the one-fourth inch pieces into the beaker. If the samples are accurately weighed, the abundance of silicoflagellates in the fossil plankton can be quantitatively determined. (Tappan *et al.*, 1971)

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[2] In this paper we use the term "sulfuric acid fuming" in the sense of concentrated H_2SO_4 which is boiling and decomposing to H_2O and SO_3 white fumes. We do not mean "fuming sulfuric acid" which is $H_2S_2O_7$ (H_2SO_4 with SO_3 in solution).

Cover the sample in the 1000-ml. beaker with about 300 ml. of distilled water. Large beakers are needed because some chemical reactions can be quite vigorous.

If a worker is familiar enough with the microorganisms in his source of tap water to recognize them as contaminants, if the mineral content of the tap water is relatively low, and if the expense of distilled water is a factor to be considered, then good tap water filtered at the tap could be used in many of the steps described in this paper.

3. Place a heavy watch glass over the beaker and add concentrated, chemically pure hydrochloric acid (HCl) through the lips in increments of 10 ml. if effervescence is evident. If there is no effervescence, slowly add all 100 ml. at one time. However, care must be taken because if the sample has a high calcium carbonate ($CaCO_3$) content and if a large amount of hydrochloric acid is added at once, excessive effervescence might result in a spill-over.

Calcium carbonate will neutralize the acid, hence more HCl may be needed to insure an excess of acid after all the $CaCO_3$ has reacted.

If chemically pure acids are not available to the worker and if expense is a factor in the operation, then technical quality acids could be used. However, because the chemically pure acids have smaller amounts of impurities their use is recommended.

The purpose of the HCl treatment is to dissolve oxides and salts (other than silicates) of metals whose chlorides are soluble. These soluble chlorides can then be decanted away. This treatment will work for most metals except silver and lead in the marine sedimentary rock sample.

4. Place the beaker on a hot plate and keep the solution at a gentle boil until all reactions have ended. Suggested hot plate dimensions are: top, three-eighths inch thick, good quality stainless steel, 20 × 20 inches; 10-inch-high stainless steel legs; and 1½-inch-high removable stainless steel railing to prevent beakers from falling off the edge. Heat should be supplied by two or three Meker burners. Rubber tubing should not be used because of the high heat. Tygon or other heat resistant tubing should be used, or, better yet, a direct connection with steel piping with valve control.

Add distilled water to the beaker until half-filled, set aside until no microfossils are suspended in the solution. This should be determined by examination of a drop of the solution under the microscope at 100 ×.

Decant carefully so as not to lose any of the microfossils. Fill the beaker half full with distilled water. Cover with a watch glass and again bring the solution to a gentle boil. More soluble chlorides will now be taken into solution as will be indicated by the color of the water. Boil gently for at least an hour, cool, and examine a drop of the solution under a micro-

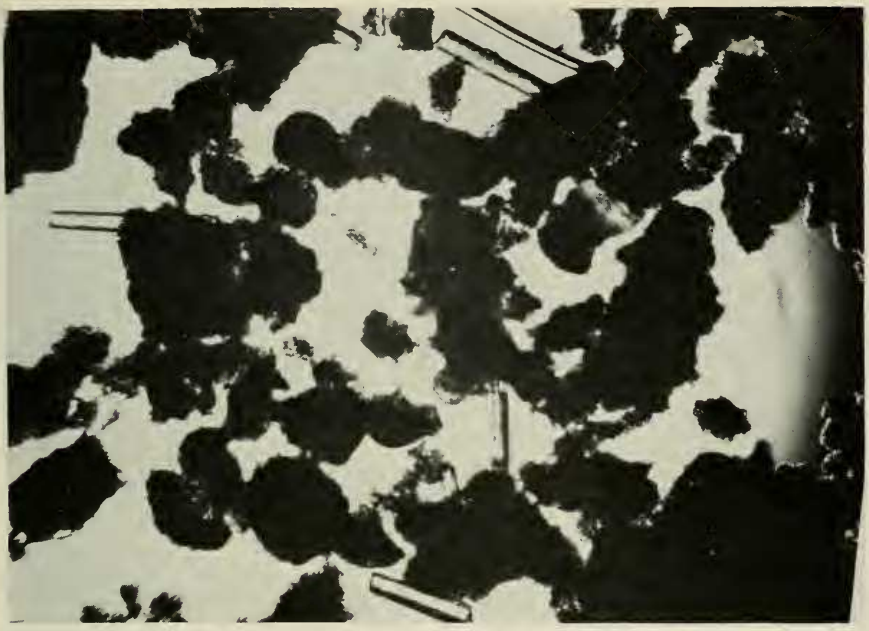

FIGURE 1. Sample broken down with HCl and water, retained after washing on 400-mesh screen.

scope at 100 ×. If there are no microfossils in the solution, decant. If microfossils are still in suspension, wait until they settle, then decant.

5. Add distilled water to the beaker until half-filled. Remove the fine sediment by vigorously swirling the solution in the beaker so that the fine fraction (i.e. the decomposed and disaggregated material of the rock sample) goes into suspension. Then immediately pour off the solution with its suspended matter into another clean 2000-ml. Pyrex beaker marked with the sample number and an 'A', leaving the coarser material in the original beaker.

   Repeat step 5 several times until the solution in the original beaker is neutral to litmus paper.

6. To the original beaker containing the hydrochloric acid-treated sample that is not yet decomposed and not yet disaggregated, add a solution of sodium carbonate Na_2CO_3 (50 grams per liter of distilled water) until the sample is covered with the solution.

   Heat slowly on the hot plate and gradually add a 30 percent solution of chemically pure hydrogen peroxide (H_2O_2). This must be done carefully, a few drops at a time, because the reaction could be quite vigorous and some of the sample could be lost. Continue this careful addition of

FIGURE 2. Sample fumed with H_2SO_4, washed and retained on 400-mesh screen.

hydrogen peroxide until 10 ml. have been added. Boil very slowly for 30 minutes. Then remove the beaker from the hot plate to cool and allow the sediment to settle.

The boiling with hydrogen peroxide helps to disaggregate the particles in the sample and also to oxidize the organic matter. The organic matter in the sample probably is the residue of plankton, seaweed, etc., partially decomposed and combined with other materials desiccated and oxidized while at outcrop. If the sample has not been exposed to the atmosphere (e.g. a deep-sea core sample), it will not be oxidized and desiccated; the organic matter will usually go into solution easily and chemical extraction of microfossils is simple. This is why we stated earlier that most deep-sea cores are relatively easy to prepare.

7. If there are no microfossils in suspension, decant and discard the solution which is in beaker 'A'. Test for acidity with litmus paper. If acidic, wash several times and decant until the solution is neutral. Examine a drop of the solution with the microscope at 100 × before each decantation to be sure that no microfossils will be lost.

8. Decant the sodium carbonate - hydrogen peroxide treated fine sediment of step 6 into beaker 'A'. Wash several times by swirling the original beaker until the fine disaggregated and decomposed fraction of the sample is in suspension. Continue washing and decanting until beaker 'A' is nearly filled.

FIGURE 3. Fumed sample treated with NaOH and H_2O_2, washed and retained on 400-mesh screen.

Then set beaker 'A' aside for the suspension to settle. Decant when the solution above the precipitate in beaker 'A' is clear.

Place the original beaker which contains the undisaggregated material back on the hot plate and add about 400 ml. of distilled water. Bring to boil and keep at a low boil for about three-quarters of an hour.

9. In most cases the sodium carbonate and hydrogen peroxide boiling at this stage should have decomposed or disaggregated most of the remaining cementing agents which have held the rock sample together. The boiling water in step 8 should have completely disaggregated the sample.

However, there are samples that will not react, or will barely react, to this treatment. In such a case, after decanting the sodium carbonate solution into beaker 'A' cover the remaining undecomposed and undisaggregated sample in the original beaker with a solution of sodium hydroxide (20 grams NaOH to 1000 ml. of distilled water) and carefully add 1 ml. of 30 percent chemically pure hydrogen peroxide (H_2O_2). Bring the solution to a boil and continue the boiling for five minutes. Remove from the hot plate, cool and decant the solution and fine sediment into beaker 'A'. Add 400 ml.

of distilled water to the remaining residue in the original beaker and boil for 30 minutes at which time the sample should be completely disaggregated. Remove from the hot plate, cool, swirl the solution, and decant the suspended matter and solution into beaker 'A'. If at this stage undecomposed material still exists in the original beaker, dry it overnight in an oven at 100° C. Label it 'Partially Treated' and store it in a container that also clearly records its locality data.

10. All the fine material that has been separated from the larger pieces of the marine sedimentary rock sample is now in beaker 'A'. Fill this beaker with distilled water and allow the solution to settle. Examine a drop of the solution under the microscope at 100 ×. If there are no microfossils in suspension, decant and discard the solution.

If in the acid treatment all of the metal ions have not been removed, they will now exist as reprecipitated carbonates or hydrates. To remove them, add 100 ml. of concentrated chemically pure hydrochloric acid (HCl) and 400 ml. of distilled water, cover with a watch glass and boil for one hour or more. Remove beaker 'A' from the hot plate, allow it to cool and allow the sediment to settle. Examine the suspended matter in the solution. If the microfossils have settled, decant and discard the solution. Continue to wash the sediment by repeated decantations until the solution is neutral, making sure that no microfossils are poured away.

11. The purpose of this step is to reduce the bulk of the sample. Use a clean 2-inch-deep, 12-inch-diameter, 400-mesh all stainless steel screen, and a clean 10-liter bucket to retain the screened material. Before screening the material, wet the screen on both sides with water; if this is not done water will not pass through this fine mesh.

Add water to beaker 'A' until about half full, swirl the solution in the beaker until the fine sediments are in suspension. Allow the heavier particles to settle: a minute should be sufficient time. Then pour onto the screen a little of the suspended material while agitating the screen by gently striking it on the side with the palm of the hand. To aid the screening, use a plastic wash bottle so held that water will flow onto the screen. The plastic wash bottle should have plastic tubing in order to protect the expensive screen against accidental contact with the tubing. Continue agitating and washing the material on the screen until the water passing through carries no more sediment. Do not use your fingers or any tool to force material through the screen as this will damage both screen and sample. Tilt the screen slightly and wash the retained material to the edge of the screen. With the wash bottle, carefully wash the retained material on the screen into the beaker to be used for the sulfuric acid fuming step. The beaker size should be either 400 or 250-ml. depending upon the amount of

residue remaining on the 400-mesh screen.[3] Continue this screening process until all the sample that is being treated has been washed on the 400-mesh screen and transferred to either the 250 or 400-ml. beaker.

12. Because the screen openings of the 400-mesh screen are 38 microns in size, some small silicoflagellates and other small microfossils will pass through these screen openings. These microscopic fossils should be retained for study. However, it has been our experience that the −400 mesh fraction frequently does not contain many silicoflagellates. If there are no microfossils in suspension, decant the solution from the 10-liter bucket, dry its sediment overnight in an oven at 100° C., label, and store for examination and study.

The +400 fraction which does not go through the screen will have undecomposed fragments of the sample containing fossils that are smaller than 38 microns. However, after completing all the steps these remaining specimens, smaller than 38 microns in size, will be freed and available for study.

Clean the screen after use with a surfactant solution. If possible use an ultrasonic cleaning device.

13. In this dangerous step the sample is boiled in sulfuric acid in order to dissolve organic cementing agents.

After the microfossils have settled to the bottom of the 250 or 400-ml. beaker, decant the water. If the layer of the sediment is more than ¼-inch thick, use a 400-ml. beaker.

Extreme caution should be used while the sample is subjected to the sulfuric acid treatment and a face mask and eye goggles should be worn. Place a heavy watch glass over the Pyrex beaker and with the aid of a pipette to which a rubber bulb is attached, slowly add small increments of chemically pure concentrated sulfuric acid through the lip of the beaker. These small amounts of acid should be run down the side of the beaker rather than added directly to the contents of the beaker. Agitate the beaker occasionally as the acid is being added in order to avoid any vigorous action. Continue the addition of sulfuric acid slowly until at least 150 ml. has been added, and the sample is covered with at least one inch of sulfuric acid. Rotate the beaker in an arc with the aid of beaker forceps to mix the acid with the contents of the beaker.

Place the beaker on a ring stand over a Nichrome wire gauze and adjust the Meker burner so that it is about two inches below the wire gauze. This system is necessary because the previously used hot plate will not produce temperatures sufficiently high for this step.

The beaker lip should be kept pointed away from the worker.

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[3] The material that is retained on the 400-mesh screen is called the +400 fraction, and is read as 'plus' 400. Similarly, that which passes through the 400-mesh is called the −400 fraction, and is read as 'minus' 400.

If the contents start to bump, hold the beaker with the beaker forceps and swirl the solution with its sediment. This should minimize the bumping. If the solution becomes viscous, remove from heat and slowly and carefully add more sulfuric acid. Then continue heating. If the solution should start to foam excessively, slide the watch glass partially off the top of the beaker so that the solution is exposed to the air and remove from heat. As soon as the foaming has subsided, heat again. This tendency to foam generally lasts only a short time. **Never** add water to prevent foaming, because a violent reaction would take place. After white fumes appear, continue heating for about ten minutes. Then put the solution aside for about two hours until the beaker has cooled to room temperature.

14. With the aid of a pipette to which a rubber bulb is attached, slowly and carefully add 10 ml. of concentrated chemically pure nitric acid (HNO_3) to the fumed sample through the lip of the beaker which is covered with a watch glass, and again bring the solution to fumes over the Meker burner. The combination of the two acids will oxidize the sample and change the color of the sediment to white. Set this solution aside to cool to room temperature. Through the lip of the beaker which is covered with a watch glass carefully add small amounts of distilled water down the side of the beaker, a few drops at a time. Swirl the solution with beaker forceps after each addition of water until there is no reaction.

15. Fill the beaker three-fourths full with water and allow to settle until the solution above the sediment is clear. At this point do not examine the solution under a microscope because of the high acidity.

Decant carefully and discard the solution making sure that none of the sediment is lost. Fill the beaker three-fourths full with distilled water. Swirl the solution in the beaker as water is added to insure that the sample is being washed. Allow the sediment to settle and examine it under a microscope, as in earlier washings, for the presence of microfossils. Continue the washings, examinations, and decantations until the solution gives a neutral reaction to litmus paper.

16. Add 100 ml. of approximately 1.0 N solution of sodium hydroxide[4] (40 grams of sodium hydroxide per liter of distilled water) and two ml. of a 30 percent solution of hydrogen peroxide to the solution. Place the beaker on a ring stand over gauze and adjust the flame of the Meker burner to give a low heat.

Continue to use extreme care. Gently rotate the beaker holding it with beaker forceps in such a way that the heat of the flame contacts the outer

[4] NaOH will dissolve siliceous microfossils. Therefore a test was made in order to determine within what limits sodium hydroxide can be used. A solution containing 20 grams of sodium hydroxide per liter of water was placed in a beaker containing a sample of fossil silicoflagellates and diatoms and heated to dryness. Water was then added and the microfossils examined. There was no apparent damage to the fossils.

edges of the beaker. Care should be taken to prevent the flame from coming in contact with the interface between the solution and air, for even a Pyrex beaker might then crack. As soon as the solution becomes warm the H_2O_2 will start decomposing and bubbles of oxygen will become attached to particles of sediment in the beaker, eliminating to a great extent the hazard of bumping. Without the addition of hydrogen peroxide, bumping could be severe enough to cause loss of most of the contents of the beaker. Allow the solution to boil for two minutes. Remove from heat and allow the sediment to settle so that the solution above the sediment does not contain any microfossils in suspension.

The purpose of this sodium hydroxide treatment is to decompose the siliceous fraction of the sample that is cementing the sediment together and to decompose those siliceous particles that are adhering to the microfossils.

17. Decant the solution and add 20 ml. of HCl. Cover the beaker with a watch glass and bring the acidified solution to a boil on the hot plate and continue at a gentle boil for about 30 minutes. Remove from the hot plate and cool. Decant after the sediment and microfossils have settled. This last decantation may be used to determine qualitatively what metallic ions are left in the sample.

Add distilled water to the beaker, allow microfossils to settle, then decant, using the prior described precautions of not decanting any of the sample. Test for acidity. If acidic, wash again by decantation until the solution is neutral to litmus paper. The sample should now be completely clean.

18. Nest three stainless steel screens 150, 250, and 400-mesh. This will yield four fractions: +150, –150 +250, –250 +400, and –400-mesh. Such a division of the treated sample makes it possible to study specimens more effectively as well as to make better slides, because smaller specimens will not be obscured by larger ones. Pour the sample onto the top of the 150-mesh screen of the nested screens, which in turn are placed on top of a 2000-ml. Pyrex beaker. The lip of the beaker will serve as an air vent. Wash each screen well with a wash bottle until little or no sediment passes through the screen. When no more sediment will pass through the 150-mesh screen, tilt it and carefully wash the sample with the wash bottle into a beaker marked with the sample locality description and '+150-mesh'.

As the screening proceeds, check to see that the volume of water is not increased in the 400 and 250-mesh screens to a point of overfill. If this occurs, place the top two screens onto another clean, labeled 2000-ml. beaker and agitate the 400-mesh screen by striking it gently with the palm of the hand until it no longer contains water. Then place the 250-mesh screen on top of the 400-mesh screen and continue the process until the sample has been thoroughly washed and all the sediment transferred to properly marked beakers with distilled water.

Prepare the bottles in which the four samples will be kept by washing the bottles with distilled water, and attaching labels giving the locality data and the appropriate mesh number data (e.g. +150, –150 +250, –250 +400, and –400-mesh).

Allow the fraction of the sample which has passed through the 400-mesh screen to settle for at least two hours. Examine for microfossils in suspension. If fossils are in a drop of the solution, wait until they have settled. Then decant and carefully transfer this fraction to its bottle. Preserve each sample with the addition of four drops of 37 percent solution of formaldehyde in bottles of approximately 30 cc. capacity. Fill bottles about three-fourths full with distilled water. Use leak-proof caps.

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